GEIS Gazette

Issue 4 May 2005

MARBURG EPIDEMIC PREPAREDNESS IN KENYA

Rosemary Sang

Following the reports of a massive outbreak of Marburg Virus currently going on in Angola, the Kenya Ministry of health formed a National Taskforce Committee. The taskforce is to put in place surveillance and national preparedness activities to prevent possible transfer of the virus through travelers arriving from the epidemic zone. The epidemic is still on and official reports from the World Health Organization (WHO) puts the total number of cases as of 17th May 2005 at 337 cases, 311 deaths; a Case Fatality Rate (CFR) of 92%. This is the worst ever outbreak of Marburg Virus.

Several subcommittees were set up including clinical, surveillance, logistics and laboratory subcommittees. The Kenya Medical Research Institute (KEMRI) was mandated to set up the laboratory subcommittee together with Centers for Disease Control and Prevention (CDC) and the Walter Reed Project (WRP).

The committee has met several times with representation from KEMRI, CDC, WRP, WHO, Kenyatta National Hospital, National Public Health Laboratories, laboratories of major private hospitals in Nairobi including: Mater, MP Shah, Aga Khan and Nairobi Hospitals.

Marburg, like Ebola, Crimean-Congo Hemorrhagic Fever and Lassa Viruses is notorious for secondary transmission in health care settings and homes. Transmission of the virus to unsuspecting health care workers and relatives through direst unprotected contact with infected patient secretions and fluids is the main route of transmission.

A number of strategies and activities have therefore been agreed upon during the deliberations. These activities will constitute response preparedness plans in the event of a potential situation of infected travelers arriving in the country from the outbreak zone and posing a risk of transmission. They currently include:

1. CDC and WRP will jointly provide some protective gear for response by the laboratory staff from KEMRI. More protective gear is expected from the Ministry of Health.
2. The VHF laboratory has in place the necessary reagents and staff capacity to carry out antigen detection assays by ELISA and RT-PCR after collecting and inactivating sample on site. A portable size water bath will be provided by WRP for onsite inactivation of sample for ELISA.
3. CDC offered to provide training to health staff onsite (health facilities) on safety and how to respond. The training activities will be rolled out soon starting with the hospitals in Nairobi.
4. A check list of actions and requirements for emergency response for the KEMRI lab staff, together with a flier of safety guidelines for health facilities has been developed by the committee to make the response plans more complete.

The tasks are expected to minimize transmission in likely health facilities and avert tragic consequences.

It is however felt that there is need for KEMRI to continue hosting the VHF epidemic preparedness committee as a continuous activity considering that the country falls within the hotspot of VHF epidemics in Africa and that cases of Marburg virus infection have been detected in Kenya in the past. The need for constant preparedness in this region cannot be over emphasized.

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Walter Reed Project
GEIS Department
P.O. Box 606
Nairobi 00621
Tel: 254-20-2729303
Fax: 254-20-2714592

Malindi Site Overview

Jeremiah Kambi

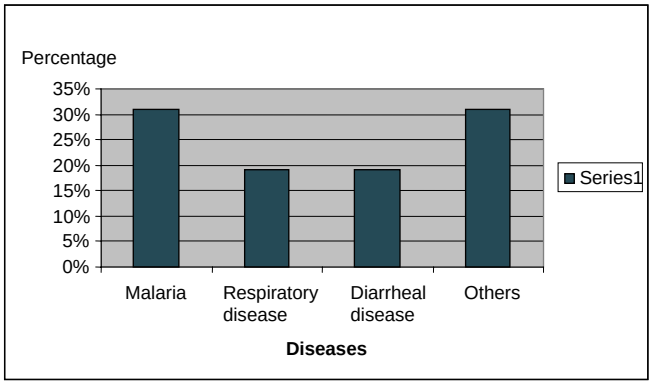
The Malindi GEIS satellite site is stationed within the Malindi District Hospital. Malindi District is a low-lying coastal town at a latitude of 3º 13"S and 40º 17"E. It is a cosmopolitan town whose local population is very diverse.

The district hospital serves as referral hospital to the 67 health facilities in the district. There are three Health Centers located in Gede, Marafa and Gongoni. The remaining facilities are private hospitals, government and privately owned clinics and dispensaries.

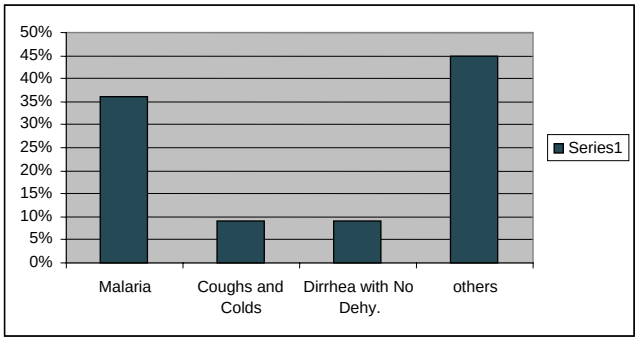
The population of Malindi District according to the Malindi District Statistics Offices stands at 355,782 persons.

Malindi District has several endemic diseases. The causes of morbidity according to records from the District Health Information Systems for the year 2004 are as follows:

Causes of Morbidity in Adults



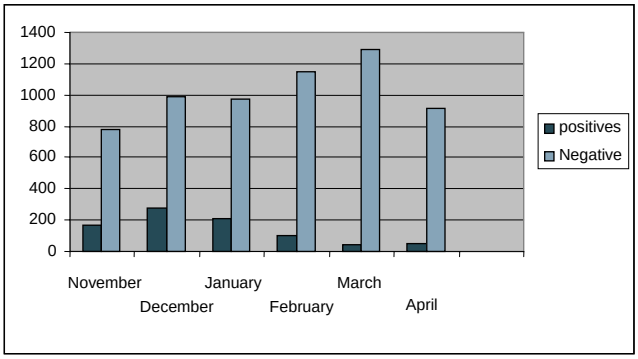
Causes of Morbidity in Pediatrics Cases



The major cause of morbidity in the district is malaria. It accounts for 31% of all adult cases and 36% of cases in children. Respiratory diseases and diarrheal illnesses are the second and third leading causes of morbidity respectively in both populations.

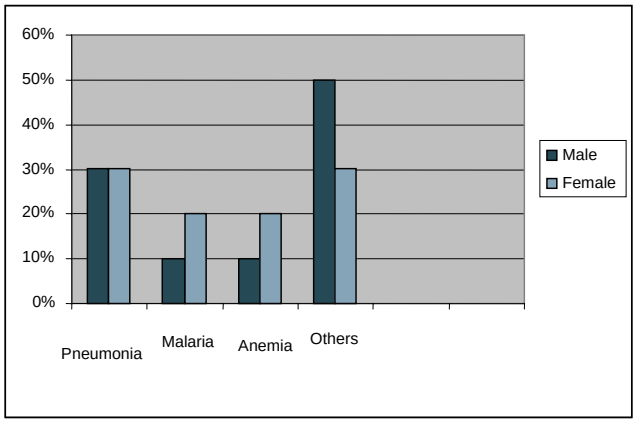
Below is a chart showing the incidence of malaria in the last six months. A comparison of smear positive and smear negative cases in indicated.

Comparison of malaria cases by smear reading

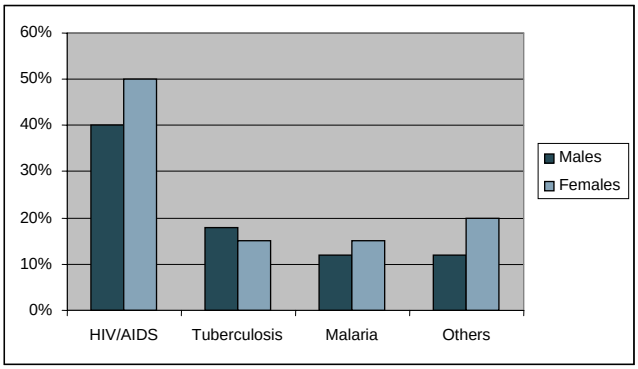


Causes of mortality in the district are significantly different for adults than they are for children. Adult mortalities are mainly attributed to HIV/AIDS and Tuberculosis; children are more susceptible to pneumonia and malaria. The cases of death are also differentiated by sex and are indicated in the graphs below:

Causes of Mortality in Pediatrics Case by Sex



Causes of Mortality in Adults by Sex



The Walter Reed Project, led by the GEIS Coordinator, first arrived in Malindi in the year 2002. By January

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2003, WRP had finished renovating a residential house for its office and put a new roof to the main lab that was leaking. All will agree it is after this beautiful work that the hospital embarked on a systematic, well coordinated renovation that has changed the face of the hospital.

Currently, we are working on three protocols. The perennial Acute Febrile Illness Study is the oldest and has so far enrolled samples, from November 2002. The Malaria Drug Sensitivity Study – which is a follow-up of the 100 samples collected in the year 2004 – has contributed immensely to the change of government policy in treating malaria countrywide. Lastly is the long awaited Study of Diarrhea Illnesses that commenced in May 2005.

The Malindi GEIS satellite site is stationed within the Malindi District Hospital and serves as referral hospital to the 67 health facilities in the district.

The Malindi site has two members of staff: a clinical officer, Mr. Jeremiah Kiponda who graduated from the School of Clinical Medicine – Port-Reitz with a diploma in Clinical Medicine and Surgery. The laboratory department is headed by Ruth Sarah Mupa who has certificate in Laboratory Technology from Mombasa Polytechnic. Both are trained in outbreak investigation, laboratory safety and computer usage. Ruth is also trained on the handling of biohazards and transport of biomedical materials.

In conclusion, the work taking place at the Malindi District Hospital is a wonderful collaborative undertaking that is invaluable. The Acute Febrile Illness Study is on a path to find out of there is something that may be hitherto undiscovered – such documentation may be vital in preventing unexplained morbidity and mortality in the region.

The other on-going studies are targeting two of the three major causes of mortality for study; the information received from the work done here is very useful. Most changes of policy in the approach and management of malaria, diarrhea and respiratory diseases may be attributed to WRP activities in Malindi and other parts of the country and we hope these results will be extrapolated to other areas and persons. It is vital to acknowledge the contribution by the hospital towards our continued endeavors.

THE EFFECT OF CYCLIC ADENOSINE MONOPHOSPHATE (cAMP MODULATORS ON THE ACTIVITY OF SELECTED ANTI-MALARIALS

Julia Wangui

Malaria is one of the most prevalent human infections worldwide resulting in 1.5million to 2.7million deaths every year. The fight against the disease involves chemotherapy, vector control and vaccine production.

The evolution of multi-drug resistance raises fear that emergence of resistance to anti-malarials may proceed faster than the development of new and effective drugs; therefore, there is a requirement to get ways of improving the efficacy of the available anti-malarials. Optimization of therapy with available drugs including the use of combination therapy is one of the efforts being made to achieve this. An appropriately chosen combination must at least be additive in potency and might provide synergistic activity

Cyclic nucleotides are second messengers of intracellular events initiated by activation of many types of hormones and neurotransmitter receptors. Cyclic adenosine monophosphate (cAMP) is produced following a cascade of events leading to stimulation of adenylyl cyclase. There is a drastic rise of cAMP in the erythrocyte during malaria infection.

This study aims at demonstrating the effect of cAMP modulators on selected anti-malarials.

Chloroquine, Mefloquine, Quinine, Amodiaquine and Doxycycline are tested in combination with 2 adenylyl cyclase activators and 2 adenylyl cyclase inhibitors against two *P. falciparum* strains (D6 and W2). These isolates are maintained in 25cc flasks in an atmosphere of 91% nitrogen, 3% oxygen and 6% carbon dioxide. The culture media is supplemented with 10% pooled serum and 0 positive blood cells. Susceptibility testing is done using semi-automated micro-dilution technique. Each drug is tested alone and in combination with a modulator at fixed ratios in a 96 well micro titer plate. The suspension of the drug and parasites are incubated at 37ºC for 24 hrs before adding the radio-label (^{3}H- hypoxanthine) and a further incubation for 18 hrs. The inhibitory concentration (IC_{50}) of each drug and the drugs in combination is determined and 50% fractional inhibitory concentrations (FIC_{50}) calculated using Oracle database software. Sum FIC greater than 1 will be an indication of antagonism, less than 1 an indication of synergism while equal to 1 additivity.

This study will help in identification of the combinations showing additivity or synergy which will assist in finding ways of augmenting the already existing anti-malarials to combat the resistant parasite.

Standardization of SOP Format at the GEIS Facilities

Paulomi Patel

Since the inception of the Quality Assurance Program at USAMRU-K, the GEIS laboratories have been working relentlessly to achieve the QA goals. Consequently, the GEIS laboratories have seen some major changes in the quality of work over the past few months. Some of these changes have come through a better understanding of the importance of QA as a paramount essence leading to high performance and professional rigor, preventing laboratory mistakes and achieving significant improvements in testing performance.

To support this endeavor all the GEIS facilities have gone through an entire year of updating and changing Standard Operating Procedure (SOP) formats. They are currently amidst amending them from the previous format to a new version as outlined by the 'Standard for Preparation, Implementation and Maintenance of Standard Operating Procedures' – USK 001 V1. This SOP describes the preparation, implementation and maintenance of all SOPs within United States Army Medical Research Unit – Kenya (USAMRU-K). All the SOPs prepared using these guidelines will also be in accordance with the Medical Research and Materiel Command Standards for SOPs. Furthermore, these standards ensure compliance with FDA, DOD regulations, MeRITS and GCP requirements as appropriate for all investigational trials conducted within not only GEIS facilities but also USAMRU-K as a whole.

These SOPs are a set of written instructions that document the routine or repetitive activity followed by the laboratories. The development and use of SOPs has become an integral part of the successful quality system at all the GEIS facilities. They provide the personnel with the information to perform their job properly, and facilitate consistency in the quality and integrity of the end result.

Frequent training sessions are conducted where personnel at the GEIS facility in Nairobi are tutored on preparing, maintaining and implementing SOPs for their laboratories. The GEIS Satellite Sites personnel have also been trained on the proper use of these SOPs. To counteract variability of results

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MOLECULAR CHARACTERIZATION OF YELLOW FEVER VIRUS ISOLATES FROM IMATONG SOUTH SUDAN IDENTIFIES A SUB-LINEAGE IN E-GENOTYPE I.

Clayton Onyango

Following an outbreak of Yellow Fever Virus in Imatong South Sudan in 2003, comprehensive phylogenetic studies have been performed to understand the genotype of the virus that caused the outbreak. Five patient samples that yielded isolates were investigated by comparison of the nucleotide sequence of the Envelope Protein Gene (E) of these isolates and other 17 isolates previously analyzed.

Several isolates from Africa had previously been analyzed and 'uniformly weighted parsimony algorithm analysis' used to define two major evolutionary Yellow Fever lineages designated E genotypes I and II. The E genotype I contains two sub-lineages, IA and IB viruses isolated from East and Central Africa. E genotype II viruses are also divided into two sub-lineages; IIA viruses from West Africa and IIB viruses from South America.

Several isolates from Africa had previously been analyzed and used to define two major evolutionary Yellow Fever lineages

Unique signature patterns have been identified at both nucleotide 111 and amino acid number 12 within Yellow Fever Virus E gene. Yellow Fever viruses from East and Central Africa contain a unique signature at nucleotide 60 and amino acid number 5; those from West Africa contain such signatures at nucleotide 25 and amino acid number 2.

In this study, a sub-lineage has evolved in E-genotype I hence it is further classified into E-genotype IA and IB. Further explanations on the timing of the outbreak in a 'virgin' population of Imatong have also been postulated. The fact that unique signatures have been found with the E- gene of isolates that caused an outbreak in Kenya in 1993 as well as those that caused an outbreak in Imatong 2003 coupled by negligible transversion and transition amongst these isolates could help explain the magnitude and timing of the outbreak. Other factors like civil unrest and rainy season also contributed to the timing of the outbreak.

The main aim of the study therefore was to characterize and analyze these isolates based on the entire E gene. The isolates were exposed to RT-PCR, gene cloning, plasmid preparation followed by

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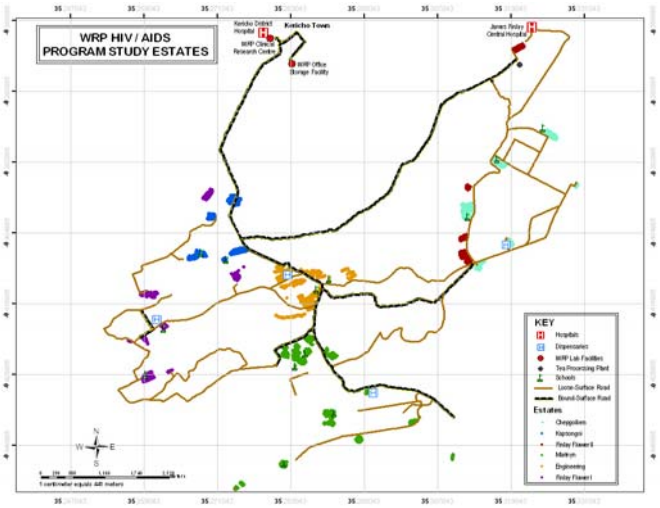
Use of Geographic Information Systems in Epidemiology

Carol Tungwony

Geographic Information System (GIS) use in the area of public health is very low. The system developed faster in areas such as transportation, law enforcement, monitoring geological and climatic phenomena around the world among others. It is in the recent past that GIS is gaining momentum in its use in the area of epidemiology and public health.

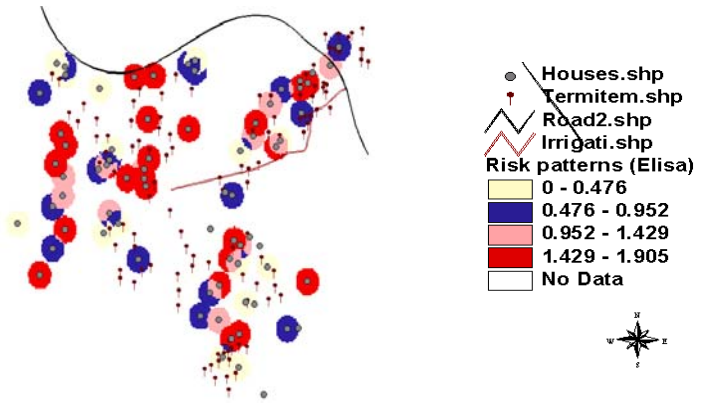
Some of the most common uses of GIS in epidemiology include: the determination of the health situation in a geographical area, generation and analysis of research hypotheses, identification of high risk health groups, planning and programming of epidemiology activities and monitoring and evaluations of interventions.

At USAMRU-K, GIS has been applied to various protocols such as mapping the study area of HIV Program in Kericho tea plantations with the following aims: to determining the population dynamics to facilitate success in enrolment of volunteers participating in the study, to determine the locations of all study volunteers to improve follow up programs and facilitate activities of fieldwork and ease division of labor among the field coordinators and fieldworkers.

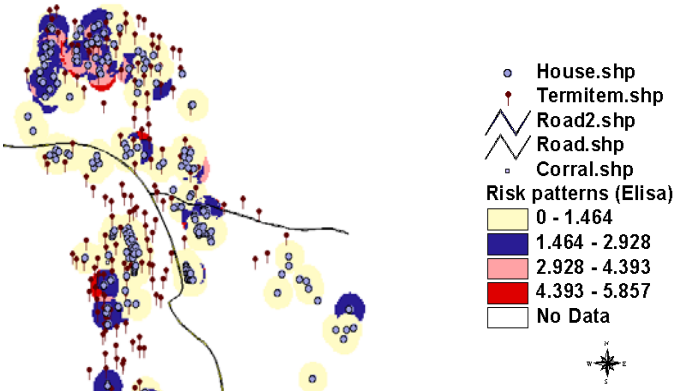


GIS was used to map risk patterns of Visceral Leishmaniasis in Baringo. The study compared two villages that are economically different. Risk patterns are conclusively drawn below.

Parkarin Village



Loboi Village



GIS represent a powerful tool that supports health situation analysis, operations research, and surveillance for the prevention and control health problems. Moreover, these systems provide analytical support for the planning, programming, and evaluation of activities and interventions in the health sector.

GIS is especially useful in vector borne disease research as it maps the environmental characteristics that accompany vector to allow the prediction of the distribution and risk areas for the disease. GIS can thus be considered part of decision – support systems for people who formulate and reinforce health policy.

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genetic analysis using a gene sequencer. Phylogentic analysis of the gene from these isolates was performed using ClustalW, BioEdit, Mega2, DNASIS and treeview. The reiterated UPGMA phylogenetic distance tree was exposed to a statistical analysis using bootstrap value of 100 with similar replication folks.

Supporting statistical data of the similarities amongst genotypes suggested a sub-lineage amongst the E-genotype I. Analysis of the amino acid changes observed in the signature sites were predominantly very unique in sites E (46) that has a positive hydrophobicity and both alpha helix and beta sheets as secondary structures. Also site T (268) that has an Antigenic Domain A showed negative hydrophobicity with a secondary structure of both beta sheets and a turn.

Lack of sufficient sample size in the previous studies could have resulted in the classification of Yellow Fever Virus from East and Central Africa into a genotype without sub-lineage. This is attributed to the infrequent outbreaks of Yellow Fever Virus in this region as compare to West Africa, the Americas and Asia.

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generated by different laboratories conducting common processes such as malaria smears, SOPs are being developed outlining the procedures to follow for these common processes such that each site would not have to generate several SOPs on one procedure.

The development and use of SOPs has promoted quality through consistent implementation of a process or procedure within the GEIS protocols. These SOPs are also being used as part of personnel training program since they provide detailed work instructions. Successful and complete implementation of the SOPs is anticipated to minimize opportunities for miscommunication. Moreover, when historical data is to being evaluated for current use, these SOPs will be valuable for reconstructing project activities when no other references are available. In addition to that, SOPs are frequently used as checklists by inspectors when auditing procedures.

Ultimately, some of the benefits of the valid SOPs generated at the end of this standardization process are: reduction in wasted work effort, along with improved data comparability, credibility, and legal defensibility.

Attn: Sheryl Bedno

GEIS Program
USAMRU - Kenya
P.O. Box 606
Nairobi 00621

ADDRESS CORRECTION REQUESTED

SITE MAILING ADDRESS STICKER